IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re of the Application of

Stephen Michnick et al

Serial Number: 09/870,018

Filed: May 31, 2001

For: A PROTEIN FRAGMENT COMPLEMENTATION ASSAY (PCA) FOR THE DETECTION OF PROTEIN-PROTEIN, PROTEIN-SMALL MOLECULE AND PROTEIN-NUCLEIC INTERACTIONS BASED ON THE *E. COLI* TEM-1 β-LACTAMASE

Group Art Unit: 1645
Examiner: UNASSIGNED

RESPONSE TO FORMALITIES LETTER OF AUGUST 8, 2001

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

In response to the formalities letter dated August 8, 2001, it is respectfully submitted that the present application as filed does not need a sequence listing as required by 37 C.F.R. 1.821-1.825.

The present application discloses the use of fragments of a β-lactamase enzyme which enzyme is well known in the art, for purposes of conducting protein complementation assays. As it will be appreciated, the specification clearly states that the fragments used namely BLF[1] and BLF[2] correspond to residues 23-197 and 198-286 of TEM-1 β-lactamase. See Examples 1 and 2. Also note in particular the discussion starting

at page 2, line 15 to page 4, line 6.

Additionally the specification states at pages 2-4:

"...The three-dimensional structure, proposed catalytic mechanism and optimal substrates and inhibitors have been well documented. TEM-1 ß-Lactamase is a small (29 kiloDaltons) and monomeric protein consisting of 286 amino acids. The first 23 amino acids constitute a secretory signal peptide. ß-lactamases catalyses the irreversible hydrolysis of the amide bond of ß-lactam rings in penicillin or cephalosporin compounds. ß-lactamases are secreted into the periplasmic space of gram-negative strains or into the outer media by their gram-positive counterparts where they normally act. However, they will accumulate in the cytoplasm when expressed in *E. coli* or other prokaryotic or eukaryotic cells if the secreting signal peptide is genetically deleted, without effecting catalytic activity.

TEM-1 ß-lactamase meets all of the essential criteria to be an excellent candidate for a PCA strategy. Specifically, TEM-1 ß-lactamase is a relatively small, monomeric protein and is well characterized both structurally and functionally. TEM-1 ß-lactamase can be expressed in and is not toxic to prokaryotes and eukaryotes. In addition to these, unique features include that: First, ß-Lactamase is strictly a bacterial enzyme and has been genetically deleted from many standard *E. coli* strains. It is not present at all in eukaryotes. Thus, a ß-lactamase PCA could be used universally in eukaryotic cells and many prokaryotes, without any intrinsic background. Second, assays are based on catalytic turnover of substrates with rapid accumulation of product.

This enzymatic amplification should allow for relatively weak molecular interactions to be observed. Finally, the assay can be performed simultaneously or serially in a number of modes, such as *in vitro* colorimetric or fluorometric assays, or *in vivo* fluorescence or survival assays. Assays can be performed independent of the measurement platform and can easily be adapted to high-throughput formats requiring only one pipetting step.

The PCA strategy of the present invention is based on the reassembly of two rationally designed complementary fragments of TEM-1 ß-lactamase. Crystal structures of TEM-1 suggest that residues 196-200 form a loop situated outside of the core of the protein and distal to the enzymatic pocket (Figure 1). This loop is not implicated in the catalytic machinery and seems not to be important for catalysis (ref. 4). For these reasons, this site was selected to generate the two fragments. We chose to cut in the middle of the loop between residues Glu197 and Leu198. In addition, the secreting signal peptide of 23 amino acids was deleted to leave only the functional enzyme. Thus fragment [1] (BLF[1]) consists of residues 24 to 197 and fragment [2] (BLF[2]) of residues 198-286. Each of these fragments are linked to interacting domains (GCN 4 leucine Zipper or the pair of rapamycin inducible interacting proteins FKBP/FRB domain) by a linker of 15 amino acids $(Gly\text{-}\ Gly\text{-}\ Gly\text{-}\ Gly\text{-}Ser)_3$..."

Applicant has already been granted two U.S. Patents on this technology which utilizes DHFR (dihydrofolate reductase) fragments: U.S. Nos. 6,270,964 and 6,294,330.

It should be noted that in neither of those two patented cases was there a need to file sequence listings.

In view of the above, it is respectfully requested that the notice to comply with the requirements of 37 C.F.R. 1.821-1.825 be withdrawn. A copy of the formalities letter is enclosed with this reply.

Respectfully submitted,

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APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
09/870,018	05/31/2001	Stephen William Watson Michnick	ODDY 002

CONFIRMATION NO. 8430

FORMALITIES LETTER

OC000000006398921

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Date Mailed: 08/08/2001

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Applicant is given **TWO MONTHS FROM THE DATE OF THIS NOTICE** within which to file the items indicated below to avoid abandonment. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

- This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper or compact disc copy of the "Sequence Listing", as well as an amendment directing its entry into the application. Applicant must also provide a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

For questions regarding compliance to these requirements, please contact:

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A copy of this notice MUST be returned with the reply.

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